DISCOVERY

Semen characteristics of rabbits fed camel's foot (*Piliostigma thonningii*) essential oil supplemented diet

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ABSTRACT

This study aimed to investigate whether the inclusive administration of *Piliostigma thonningii* essential oil (PEO) in rabbit's diet was beneficial to their general reproductive traits. Forty-five clinically healthy weaned male Dutch rabbits of about five weeks of age were used for the experiment. The rabbits were divided into three treatment groups (T1, T2 and T3), with fifteen rabbits per group and balanced for their BW such that rabbits in each group had similar average initial BW of 0.27 ± 0.02. The experimental rabbits were administered PEO at 0, 2 and 4 mL/kg for treatments 1, 2 and 3 respectively. The semen colour was same (creamy) and semen pH was 7.00 for T1, T2 and T3. The values for ejaculatory volume varied from 0.53 to 1.03 ml with T3 having the highest value and T1 with the lowest value ($P < 0.05$). Values for semen concentration varied between 128.53 to 219.66 (x 10^6/ml) having the lowest value in T3 ($P < 0.05$) and T2 and T3 having similar values ($P > 0.05$). Values for sperm motility varied between 68.33 to 90.00% with T3 having the highest value and T1 with the lowest value ($P < 0.05$).

Keywords: Essential oil, *Piliostigma thonningii*, rabbits, semen characteristics, semen morphology, morphometrics.

1. INTRODUCTION

In Nigeria, the acute scarcity of meat due to farmer-herder crises, global pandemic and poor economic policies has compelled livestock farmers to improve feed resources utilisation, health status and meat production from their animals (Anaso et al., 2021). This shortage can be bridged by the farming of highly prolific monogastrates with short production cycles like rabbits. Rabbits possess high fertility and rapid growth rates, thereby characterizing them an excellent source of meat and protein of animal origin (Dalle and Zotte, 2002). Rabbit meat is characterized as high quality due to adequate animal protein and polyunsaturated fatty acids and low calories (El gogary et al., 2018). Tropical climate characterized by its high temperature (heat) is a major climatic factor invariably affecting rabbit farming/production. Rabbits enormously depend on respiratory evaporation for the regulation of their body temperature and this

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confers only a limited power of adaption to hot climates (Mailafia et al., 2010). Lipid peroxidation is a major problem in rabbit's production caused by oxidative stress leading to end product (meat) deterioration (El gogary et al., 2018). Furthermore, lipid oxidation adversely impacts the meat quality as it causes the formation of some toxic compounds and malondialdehyde (MDA).

The latter has been reported to negatively influence human's health (Kone et al., 2016; Cardinali et al., 2015). The recent ban on antibiotic growth promoters by several countries and the jeopardy of antibiotic resistant bacteria have compelled the search for alternatives of improving animal productivity and minimizing adverse effects on human consumers. Due to this ban, extensive research has been conducted to explore the use of phytogenics as alternate feed additives in animal nutrition. Phytogenic substances such as essential oils (EOs) are generally regarded as safe and are frequently used in the food and feed industries. The impact of EOs as a phytogenic feed substance, on the intestinal health, antioxidant status and antimicrobial activity in livestock is considered vital for the biological activities (Ahmed and Abdallah, 2020). Evaluation of semen characteristics of the animals may give some insight to the potential or the extent to which dietary treatment meets adequate reproductive standards of animals. Although some EOs have been evaluated in livestock diets with positive results, nothing or little is known about the effect of Camel's foot (*Piliostigma thonningii* Schum) EO on the productive and reproductive performance of rabbits.

2. MATERIALS AND METHODS

Experimental site

The experiment was carried out at the Monogastric Unit of the University of Abuja Teaching and Research Farm. The project site lies between latitudes $08^{0}51'$ and $09^{0}37'$N and longitudes $007^{0}20'$ and $007^{0}51'$ E. Annual rainfall ranges from 1,145 – 1,631 mm. The temperature in dry season is between 36 – 42°C and 25.8 – 30.2°C during the raining season. Relative humidity is about 60% during the raining season and 30% during dry season.

Collection of *P. thonningii* seed and the extraction of its essential oil

Piliostigma thonningii seeds was sourced from within the Guinea Savannah agro ecological zone. It was identified and authenticated by a certified taxonomist at the Department of Biological Science, Forestry Research Institute of Nigeria (FRIN). The *P. thonningii* seeds were shade-dried, finely ground and stored at room temperature until extraction. Steam distillation was the method of extraction used. The essential oil was extracted with a Clevenger apparatus using the method described by Mohamed et al., (2006); About 100 g of dried ground sample was suspended in 700 ml of distilled water, where steam distillation process was employed at 100^{o}C for about 3 hours by placing in steel apparatus. The setup was by heated up after connecting the condenser to a water inlet and outlet. This process allowed for softening of the sample and letting (discharging) the end product essential oil in vapourised form. The vapourized essential oil droplets formed and mixed with the steam (the carrier) and subsequently converged into a cooling system. Following convergence in the cooling system, the essential oil was then collected via a collection tube. The percentage of the oil content was calculated using the formulae below:

% Oil content (v/w) = volume of the oil extracted (ml) / weight of the sample taken (g) x 100%

Experimental animals, management and treatment

Forty-five clinically healthy weaned male Dutch rabbits of about five weeks of age were used for the experiment. The rabbits were purchased from National Animal Production and Research Institute, Ahmadu Bello University, Zaria, Nigeria. Two weeks prior to the arrival of the rabbits, the hutches and their immediate surroundings were cleaned (swept and washed) thoroughly and disinfected with antiseptic (Morigad) and Hypo® (sodium hypochlorite, caustic soda and de-mineralized water). All the rabbits were weighed individually using weighing scale to determine their initial BW. The animals were quarantined for two weeks and administered prophylactic treatment. The treatment included administration of anti-stress (Vitalyte®) in drinking water, subcutaneous injection with an anti-parasitic drug (Avomec®) at 0.5 mg/kg of the animal body weight (BW) for the control of endo and ecto parasites and injection with oxytetracycline HCl (a broad-spectrum antibiotic) at 1.0 mL/10 kg BW via the parenteral intramuscular route. Rabbits were also treated subcutaneously with a coccidiostat (Sulphadimidine Sodium BP solution) once at the beginning of the experiment at 1mL/rabbit according to manufacturer's recommendation. The rabbits were housed in individual open sided metabolic hutches which can separate faeces from urine.

Experimental design

The rabbits were divided into three groups, with fifteen rabbits per group and balanced for their BW such that rabbits in each group had similar average initial BW. Rabbits in the experimental groups were randomly assigned to one of three treatments in a

completely randomized design. The experimental rabbits were administered PEO at 0, 2 and 4 mL/kg for treatments 1, 2 and 3 respectively. Both water and feeds were provided ad libitum for a period of 12 weeks. Feeding was done twice a day at 08:00 hrs and 16:00 hrs. Rabbits of the control treatment were fed a basal diet as a Control diet. The diets were formulated according to National Research Council (NRC) 1984 requirement for domestic animals and the Academy of Sciences Washington nutrient requirement for growing rabbits.

Semen collection and Evaluation

The semen was collected by means of an artificial vagina filled with a warm liquid of about 45°C. The doe was fitted with this device and presented to bucks in their respective hutches (Boiti et al., 2005). Semen samples was evaluated immediately for volume by direct reading of the graduated collection vial., The result was expressed in millilitre (mL). The sample were then kept in water bath at 37°C, and the evaluations were made in sequence according to CBRA, (1998) animal manual. The appearance of the semen was determined by visualization of consistency of ejaculates and classified as: Creamy marble, creamy, thick milky, milky and watered. Smear of each semen sample was prepared, air dried, labelled and kept for further examination. The progressive motility was determined by placing 10 µL of semen into 1 mL of Tris dilution buffer (Hydroxymethylaminomethane (3.0 g), sodium citrate (2.0 g) and fructose (1.0 g)). Then a 10 µL-aliquot of the diluted semen sample was placed between preheated slide and coverslip (37°C) and evaluated under optical microscope (100 x magnification). The progressive motility was expressed in percentage.

The concentration of the spermatozoa was determined using a haemocytometer that is crossed with microscopic grids containing 25 large squares with each containing sixteen smaller squares. The total number of smaller squares on the haemocytometer was 400. Sperm cells were counted diagonally from top left to the bottom right and from top right to the bottom left in five large squares or a total of 80 smaller squares (Rekwot et al., 1997). Prior to counting, formaldehyde was used as a dilution reagent. A drop of semen was taken from each sample using automatic pipette and diluted with formaldehyde at 1:100. The haemocytometer was mounted on the microscope stage and an absorbable tube and O-no pipette was used to pipette a drop of the solution into the haemocytometer chamber. The absorbable tube and the O-no pipette was blown before pipetting to avoid air bubbles in the O-no pette. The result obtained was recorded and used to calculate the sperm cell concentration for the sample as: sperm concentration per ml × volume of ejaculate.

Sperm morphology was determined from 95 slide smears stained with 7.5% Giemsa (Doles Laboratory); the slides were immersed in the Giemsa solution for two hours, afterwards the slides were kept upright until they completely dried and viewed under the microscope to get the normal and abnormal sperm percentage. Normal spermatozoa and spermatozoa abnormalities were classified according to principles used for rabbits by Barth and Oko, (1989). The live to dead sperm ratio was estimated by the preparation of a smear of individual semen sample using eosin-nigrosin stain immediately after collection. A drop of semen sample was placed on a clean glass slide using automatic pipette. A drop of the eosin-nigrosin solution was placed alongside the semen on the slide. A gentle circular turning of the slide was done to allow a uniform mixture of the two samples. A one-quarter of the part of another clean slide was placed on top of the first sample on the slide at an angle of 45 degree to make contact with the semen sample slide and carefully drawn apart to prepare a thin smear. This was allowed to dry and thereafter labelled. This was done for each sample. After that, the slides were viewed under the microscope to count the live and dead sperm cells. The principle is that the dead sperm cells accept the stain and appear stained while the live sperm cells reject the stain and remain unstained. The procedure above was developed by Hancock, (1951).

Statistical analyses

The obtained semen characteristics data were subjected to analysis of variance (ANOVA) in a completely randomized design using SPSS (23.0). Duncan multiple range test (DMRT) of same software was used to test the significant difference between the means at ($p \leq 0.05$) level of significance.

3. RESULTS

Gross semen characteristics of rabbits fed *Piliostigma thonningii* essential oil

The ejaculatory volumes (EV), semen colour and pH, sperm motility and semen concentration are presented in figure 1. The ejaculatory volume varied significantly ($P < 0.05$) from 0.53 to 1.03 ml. T3 and T1 had the highest and lowest values, respectively, while T2 was similar to T3 and T1. The semen colour was same (creamy) for T1, T2 and T3. The semen pH was 7.00 and also same for for T1, T2 and T3.

Table 1 Gross semen characteristics of rabbits fed *Piliostigma thonningii* essential oil

Parameter	T1	T2	T3	SEM
Semen volume (ml)	0.53^{b}	0.70^{ab}	1.03^{a}	0.15
Semen colour	Creamy	Creamy	creamy	
pH	7	7	7	0.00

$^{a, b, c}$: Means with the different superscripts along the row are significantly (P < 0.05) different, T1, 0ml administration of *P. thonningii* essential oil; T2, 2ml administration of *P. thonningii* essential oil; T3, 4ml administration of *P. thonningii* essential oil.

Microscopic semen characteristics of rabbits fed *Piliostigma thonningii* essential oil

Values for sperm motility varied significantly (P < 0.05) from 68.33 to 90.00%. T3 had the highest value followed by T2 while T1 had the lowest value. The values for semen concentration varied significantly (P < 0.05) from 128.53 to 219. 66 (x 10^{6}/ml) T3 and T2 had similar values that were higher than T1 values. The live cell varied between 50.00 to 86.66 with T3 having the highest value and T1 with the lowest value ($P < 0.05$). Contrarily the dead cell varied between 13.33 to 40.00 with T3 having the lowest value and T1 with the highest value ($P < 0.05$). The values for mid piece droplet, coiled tail and detached head followed a similar trend which did not differ significantly ($P > 0.05$) among treatments. However, values for free tail and bent tail also followed a similar trend with T1 having the highest value and T3 with the lowest value ($P < 0.05$). The value for normal cell varied between 58.33 to 89.66 with T3 having the highest value and T1 with the lowest value ($P < 0.05$).

Table 2 Microscopic semen characteristics of rabbits fed *Piliostigma thonningii* essential oil

Parameter	T1	T2	T3	SEM
Progressive motility (%)	68.33^{c}	80.00^{b}	90.00^{a}	3.60
Semen concentration (10^{6})/ml	128.53^{b}	191.00^{a}	219.66^{a}	25.49
Life (%)	60.00^{b}	71.67^{ab}	76.67 ^{a}	5.61
Death ratio (%)	40.00^{a}	28.33^{b}	23.33^{b}	5.61

$^{a, b, c}$: Means with the different superscripts along the row are significantly (P < 0.05) different, T1, 0ml administration of *P. thonningii* essential oil; T2, 2ml administration of *P. thonningii* essential oil; T3, 4ml administration of *P. thonningii* essential oil.

4. DISCUSSION

Semen Characteristics

The colour of semen of experimental animals was similar for the treatments. This is in accordance to the reports of Adeyemi et al., (2014) who observed a creamy colour characteristic of semen from bucks fed supplemental diet. Good quality semen appears creamy white. Translucent semen indicates low concentration, while blood stains and unusual colour signify poor quality or contamination. The results for semen volume, motility and concentration were highest in animals administered the highest level of EO which is similar to result reported by El-Gindy et al., (2020) who fed potato peel extract (a potent antioxidant) to growing rabbits and reported an improvement in semen characteristic. Similarly, Abdel-Wareth and Metwally, (2021) who fed dietary phytogenic supplement to growing rabbits and reported that thyme essential oil (TEO) significantly improved semen characteristics of rabbits compared to the control group. Cases of lower EV could be due to frequency of collection, season and management (collectors' factor) and not necessarily due to nutrition. Variation in semen volume might also be due to differences in genetics, reproductive and health status of bucks, age of bucks, frequency of collection, pooled volume, nutrition, season and management (Soderquist et al., 1992). The higher semen volume in groups T2 and T3 was due to the administration of PEO which generally enchanced reproductive characteristics probably due to its antioxidant property in bioactive oleic acid, heptadecanoic acid and hexadecane as well as the constituents of sesquiterpenes and monoterpene hydrocarbons such as alpha-pinene and beta-caryophyllene.

The results for progressive motility and concentration were highest in animals administered the highest level of EO which is similar to result reported by El-Gindy et al., (2020) and Abdel-Wareth and Metwally, (2021). Similarly, El-Ratel et al., (2021) who fed extra virgin olive oil (EVOO), betaine (BET), and ginger (GIN), as natural antioxidants to growing rabbits reported an improvement in the progressive motility, vitality, sperm cell concentration, sperm outputs and fertility. According to Osinowo, (2016), sperm motility is related to sperm viability which is one of the indicators of sperm quality. Sperm quality could be classified as high or low. Generally motile cells are inherently viable. Osinowo, (2016) stipulated that sperm motility above 65% is considered good. Strong, progressive motility is a crucial index of the sperm viability and often seen as swirling, wave-like motions in highly concentrated ejaculates. High semen concentration is directly proportional to the increased feed intake and may be due to changes in temperature and ejaculation frequency (Ax et al., 2000). Higher sperm concentration in T2 and T3 could therefore be attributed to

the increased supply of nutrients for spermatogenic process from *P.thonningii* EO administration via the beta-caryophyllene constituent which has a potent antioxidant activity.

The non-significant difference on the semen pH was similar to result reported by Abdel-Wareth and Metwally, (2020) who reported a non-significant difference in the semen pH of animals fed thyme essential oil as compared to the control group. The results for normal and live cells were highest in the group with the highest administration of *P. thonningii* EO which followed the same pattern as Abdel-Wareth and Metwally, (2021) whose results demonstrated that thyme essential oil (TEO) increased the sperm livability as compared with control groups. Also, Abdel-Wareth and Metwally, (2021) reported that abnormal sperm was reduced as TEO increased. This is entirely similar to the result from this current experiment where the abnormal cells including the free and bent tail as well as the dead cell where higher in the control group. Administration of *P.thonningii* EO inversely increased the value for percentage live sperm, implying that it resulted positively on the live ability of sperm cells as compared to the control group. This therefore indicates that *P.thonningii* EO can be included in goat diet without any detrimental effect on semen characteristic. Cases of low percentage of live sperm have been attributed to poor performance due to under nutrition and poor- or low-quality feed (Irkham et al., 2017). Due to the antioxidant property in the *P.thonningii* EO bioactive oleic acid, heptadecanoic acid and hexadecane as constituents of sesquiterpenes and monoterpene hydrocarbons such as alpha-pinene and beta-caryophyllene the values for percentage live and normal cells where highest with the highest dose of the EO while the abnormal cells such as the bent and folded tail was highest in control groups indicating a positive EO activity.

5. CONCLUSION

The findings presented showed that the inclusive administration of PEO in the diet of rabbits was beneficial and posed no threat as shown by the significantly higher seminal characteristics or parameters such as semen volume, motility, concentration, normal cells and life cell ratio, testis length, weight, volume and epididymis head weight. This inclusion and administration of tropically extracted essential oil would be useful to further study the reproductive potential and characteristics on various animal species of different sexes and stages of development in different regions in Africa and beyond.

Informed consent

Not applicable.

Ethical approval

camel's foot (*Piliostigma thonningii*) seeds were sourced from within the Guinea Savannah agro ecological zone. The ethical guidelines for plant materials are followed in the study for experimentation. The Animal ethical guidelines are followed in the study for analysis.

Conflicts of interests

The authors declare that there are no conflicts of interests.

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Data and materials availability

All data associated with this study are present in the paper.

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